

Familial dilated cardiomyopathy

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Definition of dilated cardiomyopathy and clinical features

Dilated cardiomyopathy is a heart muscle disease of unknown aetiology¹ that is characterised by impaired systolic function of the left or both ventricles and is usually associated with cardiac enlargement. The diagnosis excludes heart muscle disease of known cause or associated with other disorders (systemic or pulmonary hypertension, coronary artery disease, valvar heart disease, or congenital cardiac abnormalities). Progressive deterioration because of symptoms of heart failure, low output state, and arrhythmia is common in dilated cardiomyopathy.

The diagnosis of dilated cardiomyopathy is based on physical and non-invasive examinations (echocardiography and possibly multiple gated angiography) and cardiac catheterisation. Investigations include ventriculography, coronary angiography (to exclude ischaemic heart disease) and endomyocardial biopsy (to exclude active myocarditis or specific heart muscle disease). The main criteria for diagnosis are depressed ejection fraction (<45–55%)^{2,3} and ventricular dilatation (left ventricular internal diastolic dimensions >2.7 cm/m² of body surface area). Earlier stages of the disease may be characterised by cardiac dilatation without detectable dysfunction in the resting state. In 5–31% of patients, however, cardiomegaly may be minimal or absent (mildly dilated cardiomyopathy).^{4,5} Mortality seems to be correlated with the degree of systolic dysfunction, whereas dilatation is not an independent predictor of prognosis.⁵

Histopathological changes are common but non-specific: they include myocyte hypertrophy, nuclear changes, myofibrillar loss, mitochondrial changes, and a variable degree of fibrosis.

Dilated cardiomyopathy is not rare: the prevalence has been estimated to be 36.5 cases per 100 000 individuals, with about 140 000 affected in the United States in 1989.⁶ Incidence in a series of studies^{6–8} was estimated to be about 8 new patients/100 000 a year with 20 000 new patients/year in the United States⁶ and an increased risk in those who are males and those who are black.^{8,9}

We have evaluated the incidence of the disease in the district of Trieste, based on post-mortem studies: this area is particularly suitable for an epidemiological survey, because there is one cardiovascular unit and routine systematic postmortem evaluation (>95% of deaths). Preliminary data indicate an annual incidence of about 7/100 000.

The prognosis in patients with dilated cardiomyopathy is considered to be poor. Morbidity¹⁰ and mortality are high. Moreover, throughout the world dilated cardiomyopathy is the chief indication for heart transplantation.² In our prospective study started in 1979 we found an increase in survival in recent years. This finding seems to be related to earlier diagnosis and better treatment.¹¹

The severity and complexity of dilated cardiomyopathy make studies of the aetiology and pathogenesis of this disease of great interest in cardiology.

Aetiology of dilated cardiomyopathy

Three main pathogeneses have been proposed during the past few years (fig 1). These hypotheses postulate an association between dilated cardiomyopathy and (a) chronic viral infection of the myocardium with consequent cell damage, or (b) abnormalities in immune function (possibly leading to an autoimmune damage), or (c) genetic factors that are directly responsible for the disease.

The viral hypothesis has been widely studied by different molecular biology methods and initially appeared to be very promising: up to 42% of myocardial tissue samples from patients with dilated cardiomyopathy were positive when tested by slot-blot hybridisation.¹² Despite the introduction of more sensitive and specific techniques, such as RNA amplification by polymerase chain reaction, the role of viral infection remains uncertain. Between 0^{13,14} and 45%¹⁵ of endomyocardial

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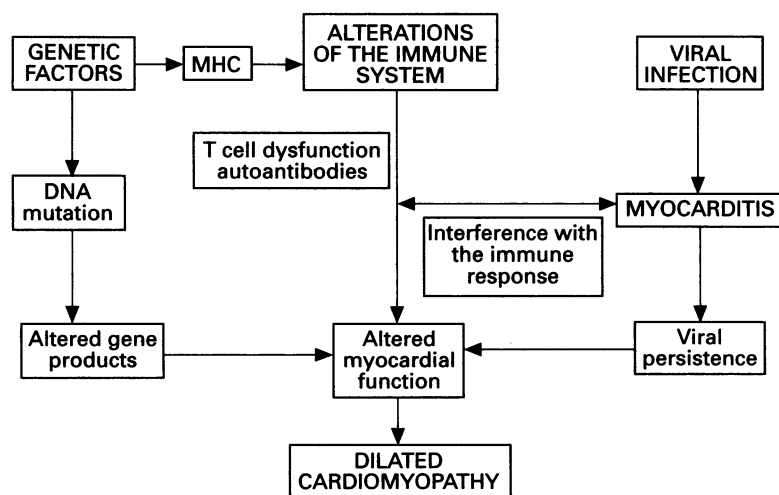


Figure 1 Hypotheses to explain the pathogenesis of dilated cardiomyopathy.

biopsy samples are reported to contain enteroviral genomes.

To clarify the broad variability of these results, we studied enteroviral persistence in endomyocardial biopsy specimens from our patients by means of a nested polymerase chain reaction.¹⁶ Taking advantage of the 5' highly conserved region of the enteroviral genome, we developed a method able to detect a single molecule of viral RNA from a broad range of enteroviral types when present in up to 1 mg of cardiac tissue. We tested 83 myocardial samples: 53 from dilated cardiomyopathy patients, 20 from clinical controls, and 10 from patients with other primary myocardial diseases. Despite the high sensitivity of this method a positive result was very uncommon (7%), suggesting that, at least in our patients, viral persistence is not a major cause of dilated cardiomyopathy.¹⁷

Several alterations of the immune system have been described in dilated cardiomyopathy. Cellular immune dysfunction was found, including decreased natural killer cell activity^{18,19} and decreased function of suppressor cell activity.^{20,21} Humoral autoimmune reactivity against myocardial tissue has also been found: this includes organ-specific autoantibodies directed against contractile proteins (α myosin and β myosin heavy chain),^{22,23} ADP-ATP carrier,²⁴ cardiac β_1 receptors,²⁵ and muscarinic acetylcholine receptor-2.²⁶ Such alterations could be the cause or the consequence of the myocardial damage. Whatever the answer they could be an important marker of disease.

The importance of genetic factors in dilated cardiomyopathy was often underestimated in the past. A review of the studies carried out during the past 10 years (table 1) shows how the frequency of the familial form (familial dilated cardiomyopathy) has increased with time. In a retrospective study at the Mayo Clinic in 1981 only 2% of patients with dilated cardiomyopathy had a familial disease.²⁷ Several more recent studies clearly show genetic transmission in more patients.

Table 1 Frequency of familial dilated cardiomyopathy

Authors	IDC study patients (No)	Familial IDC (%)	Methods
Fuster <i>et al</i> , 1981 ²⁷	104	2	Retrospective history
Michels <i>et al</i> , 1985 ²⁸	169	6	Retrospective questionnaires
Fragola <i>et al</i> , 1988 ²⁹	12	33	Prospective evaluation of relatives
Griffin <i>et al</i> , 1988 ³⁰	32	10	Study of children
Valantine <i>et al</i> , 1989 ³¹	184	9	Retrospective history
Keren <i>et al</i> , 1990 ³²	16	56	Mildly dilated cardiomyopathy study
Mestroni <i>et al</i> , 1990 ³³	165	7	Prospective study of suspect families
Michels <i>et al</i> , 1992 ³⁴	59	20	Prospective evaluation of relatives
Zachara <i>et al</i> , 1993 ³⁵	105	13	Retrospective study of suspect families

IDC, idiopathic dilated cardiomyopathy.

In a recent study at the Mayo Clinic, in which the first degree relatives of patients with dilated cardiomyopathy were systematically evaluated, evidence of a genetic transmission was found in over 20% of patients.³⁴ In a study on 165 patients we found more than 7% had a relative with a documented cardiomyopathy.³³ These data probably underestimate the real incidence of the phenomenon, because the disease can be latent or clinically undetectable or may not appear in the family history.

Genetics of familial dilated cardiomyopathy

There are no recognisable clinical or morphological differences between the sporadic and the familial forms of dilated cardiomyopathy.

In familial dilated cardiomyopathy, the pattern of transmission is mainly autosomal dominant. A single dominant locus with incomplete penetrance has been suggested in 12 families in the Mayo Clinic study.³⁴ Recessive and X linked genetic transmission have also been described.^{33,36} The possibility of polygenic inheritance (that is, traits that result from mutations in any of several different genes) have been proposed in another series of families.³⁵ Finally, mitochondrial inheritance of mitochondrial abnormalities has been found in some families.³⁷

The variability of clinical and morphological features suggests genetic heterogeneity: this means that different mutations or different genes could cause the same disease. Some families are characterised by mildly dilated cardiomyopathy.³² In other families conduction disturbances develop at the onset of the disease and cardiomegaly and heart failure develop later.^{38,39} Histological examination usually shows non-specific changes, but in some families there is a consistent pattern, such as inflammatory infiltration^{33,40} and ultrastructural abnormalities (for example, mitochondrial changes).⁴¹

Another important characteristic is the variable penetrance of the disease, namely the proportion of those affected by dilated cardiomyopathy among the carriers of the disease gene. Penetrance seems to be incomplete in most of the families³⁴ and seems to increase with age (age-related penetrance or delayed age of onset). In a series of nine families with autosomal dominant pattern of transmission, the overall penetrance was assumed to be 80% and the age-related penetrance was estimated to be 5% in those aged <20 years, 20% in those aged 20–30 years, 50% in those aged 30–40, and 90% in those aged >40 years.⁴² These data indicate that in families with familial dilated cardiomyopathy young members in particular can be carriers of the disease without any clinical manifestation.

Relatives of patients with dilated cardiomyopathy are at increased risk of the disease: 5.5% of the relatives studied by Michels *et al*³⁴ had dilated cardiomyopathy. Moreover, 9–21% of "healthy" relatives showed cardiac abnormalities, such as electrocardiographic

changes or slightly increased left ventricular dimensions in systole or in diastole.^{34,35} It is not known whether these individuals are potential carriers of the disease. Until the disease gene has been identified the answer to this question can come only from follow up studies.

Research is being concentrated on the better evaluation of the clinical, morphological, and epidemiological characteristics of familial dilated cardiomyopathy; and molecular biology now offers some new tools to study pathogenetic mechanisms.

Impact of molecular genetics on the study of familial cardiomyopathies

Because genetic diseases are caused by altered or missing gene products it is imperative to identify the "disease gene". There are two ways to do this: the first is to find the biochemical defect, recognise the gene responsible for this defect, and finally to detect the mutation causing the disease (functional cloning). The biochemical defect causing familial dilated cardiomyopathy, however, is still unknown, and direct identification of the "disease gene" is therefore impossible.

The alternative approach is so-called reverse genetics or positional cloning.⁴³ This method detects linkage of the disease with an often anonymous chromosomal locus and allows the subsequent identification of the genes encoded at that locus. This method is usually applied to large families carrying the putative disease gene, and an attempt is made to correlate the transmission of known genetic markers with the transmission of the disease. These genetic markers comprise regions of the genome that are highly polymorphic (that is, with extensive sequence variability from individual to individual).

In the most helpful circumstances each individual within a family carries two different alleles for each marker and transmits one or another of these alleles to their children. The transmission of each individual allele is then correlated with the phenotypic transmission of the disease: if transmissions of the marker and of the disease coincide the disease gene and the marker must lie in the same chromo-

somal region. A linkage study is a careful work of exclusion: the whole genome must be screened and this can require 150–200 polymorphic regions to be tested.

The probability that the genetic marker and the disease gene are linked (that is, that they lie very close in the same chromosome) is expressed as a number, called a LOD score: this is the base 10 logarithm of the odds favouring linkage. By convention, a LOD score of +3 or more (1000:1 odds) is considered as evidence of linkage while a LOD score of -2 or less (100:1 odds against) indicates no linkage. LOD scores vary as a function of θ (or recombination fraction), which is the frequency with which two loci recombine during meiosis: by calculating the frequency of recombination events, the genetic distance between two loci can be estimated.

The identification of the chromosomal location ("mapping") of the disease gene enables the subsequent study of the region, the identification and cloning of the gene, and the detection of the mutations responsible for the disease.

Several heart diseases of unknown aetiology have recently been studied by this approach (table 2). It is remarkable that different genes with different functions can determine very similar phenotypes. About half the cases of familial hypertrophic cardiomyopathy have been shown to be linked to chromosome 14, with mutations found at the level of the cardiac β myosin heavy-chain gene.⁴⁴ Other families have shown linkage with chromosome 1q3,⁴⁵ 11,⁴⁶ and 15q2⁴⁷ and preliminary data indicate the involvement of other chromosomes in other families, confirming the initial hypothesis of heterogeneity.

X linked cardiomyopathy, in particular that in the family described in 1987 by Berko and Swift,³⁶ is caused by a mutation of the dystrophin gene.⁴⁸ There is also evidence of a linkage of right ventricular dysplasia (right ventricular cardiomyopathy) with chromosome 14q23-q24 in one large Italian family.⁴⁹

There are several problems to be overcome in reverse genetic studies: in particular, the requirement for an appropriate number of sufficiently large families, including members in several generations with a homogeneous trait.

Table 2 Genetics of inherited heart muscle diseases

Disease	Transmission	Chromosomal location	Gene	Function
Cardiomyopathies:				
Familial HCM	Autosomal dominant	14q11.2-q12 ⁴⁴ 1q3 ⁴⁵ 11 ⁴⁶ 15q2 ⁴⁷	Cardiac β -MHC Unknown Unknown Unknown	Contractile
X linked CM	X linked	Xp21 ⁴⁸	Dystrophin	Cytoskeleton
Right ventricular dysplasia	Autosomal dominant	14q23 ⁴⁹	Unknown	
Secondary "cardiomyopathies":				
Duchenne muscular dystrophy	X linked	Xp21 ⁵⁰	Dystrophin	Cytoskeleton
Becker muscular dystrophy	X linked	Xp21 ⁵¹	Dystrophin	Cytoskeleton
Emery-Dreifuss	X linked	Xq28 ⁵²	Unknown	
Myotonic dystrophy	Autosomal dominant	19q13.2-13.3 ⁵³	Protein kinase	Metabolic
Mitochondrial myopathy	Maternal	Mitochondrial DNA ⁵⁷	Respiratory chain proteins	Metabolic
Primary disorders of rhythm and conduction:				
Long QT syndrome	Autosomal dominant	11p15.5 ⁵⁴	Harvey ras-1(?)	Second messenger

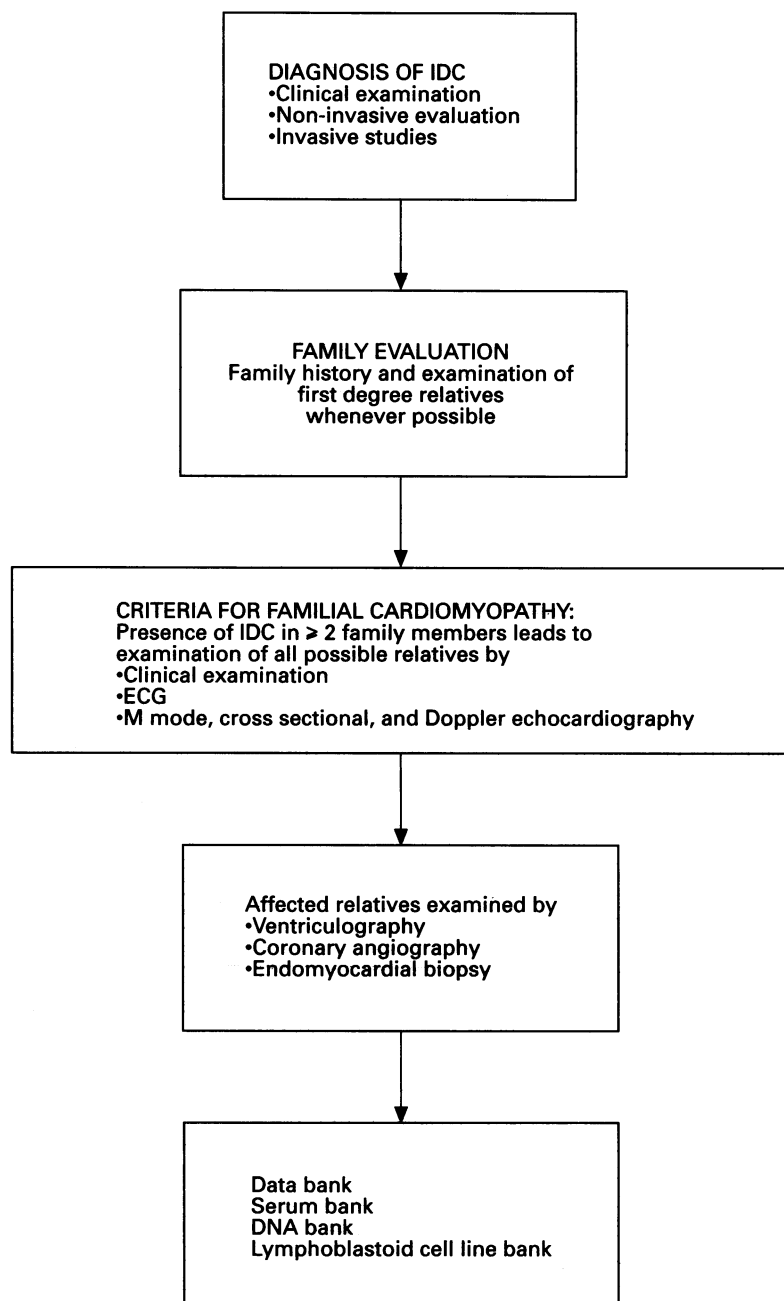


Figure 2 Protocol for the clinical and molecular genetic study of familial dilated cardiomyopathy.

Study on familial dilated cardiomyopathy
We are currently doing a prospective study of familial dilated cardiomyopathy to analyse the clinical and histological characteristics of the familial form and to try to determine the molecular basis of the disease.

Figure 2 shows the protocol of the family study. The diagnosis of dilated cardiomyopathy is established when clinical, non-invasive, and fully invasive examination, including endomyocardial biopsy, show primary myocardial dysfunction with a left ventricular ejection fraction $\leq 50\%$. Family evaluation is subsequently done by means of an accurate family history and, whenever possible, examination of the first degree relatives. If the criteria for familial cardiomyopathy are fulfilled (namely, the presence of dilated cardiomyopathy in at least two family members) we examine all possible relatives by physical examination; electrocardiogram; and M mode, cross sectional, and Doppler echocardiography. Any relative showing signs of cardiomyopathy has ventriculography, coronary angiography, and endomyocardial biopsy.

Once the diagnosis is established the clinical and investigative data on the families are collected and sera, DNA, and lymphoblastoid cell lines are stored. Lymphoblastoid cell lines, deriving from immortalisation of the B lymphocytes of the patients with Epstein-Barr virus,⁵⁵ are of great practical value in human clinical and experimental genetics: these lines provide a virtually endless source of DNA.

Twenty two families have been clinically identified so far: 14 of them are being followed up, whereas the remaining families have been excluded because of death of all affected members, remote place of residence, or unwillingness to participate in the study. We have examined 106 members (mean age 35 (range 1–63), ratio of males to females 2.2/1); 31 are affected and 75 appear to be unaffected. Table 3 shows the data.

Among the unaffected relatives is a subgroup of 12 cases of "indeterminate" or "unknown" status according to the criteria of others.^{34,35} Nine of these cases (12% of those who are unaffected) have mild echocardiographic abnormalities (slightly increased left ventricular end diastolic or end systolic dimensions standardised for age and body surface area⁵⁶) with or without electrocardiographic changes. The subgroup also includes three cases with important left ventricular dysfunction associated with systemic hypertension. None of these "indeterminate" cases had all the WHO criteria for diagnosis of cardiomyopathy.¹ The mean ages of the three groups (affected, unaffected, and indeterminate) were significantly different (table 3): with the unaffected individuals being younger than the affected and the indeterminate groups. Moreover, the proportion of those affected who were more than 30 years old (22/31, 71%) was significantly higher than the proportion who were more than 30 years old in the unaffected group (29/63, 46%, $P = 0.039$). These data support the hypothesis that, because of the reduced penetrance, some

Table 3 Clinical characteristics (mean (SD) (range)) of patients with familial dilated cardiomyopathy and their relatives

Characteristics	FDC	Unaffected	Indeterminate status
Cases (total 106)	31	63	12
M/F	24/7	27/36	6/6
Age at first symptoms (yr)	30 (12) (0–46)	—	—
Age at study (yr)	35 (13) (1–63)*	28 (18) (1–61)*	40 (25) (3–82)*
Duration of disease (yr)	6 (12)	—	—
LVEDD (mm)	63 (1)	46 (1)	54 (1)
LVESD (mm)	49 (1)	29 (0.5)	37 (1)
FS (%)	18 (8)	37 (6)	32 (9)
LVEF (%)	31 (15) (15–50)	—	—

LVEDD, left ventricular end diastolic diameter; LVESD, left ventricular end systolic diameter; FS, fractional shortening; LVEF, angiographic left ventricular ejection fraction.

* $P = 0.049$ (one way analysis of variance).

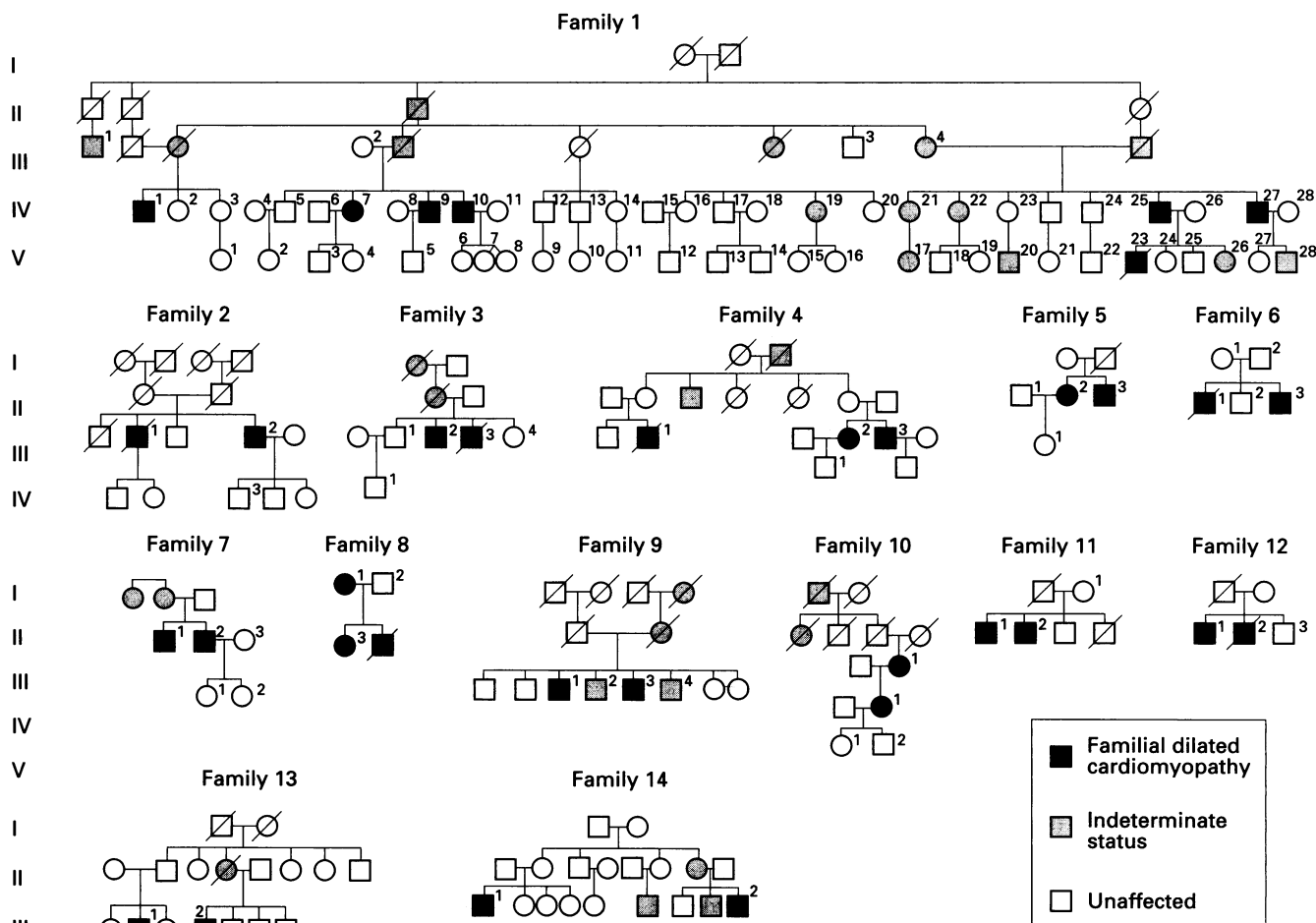


Figure 3 Pedigree of the 14 families with familial dilated cardiomyopathy.

apparently unaffected relatives may actually be carriers of the disease gene.

Figure 3 shows the pedigrees of the 14 families: nine families (1, 3, 4, 7, 8, 9, 10, 13, and 14) show an autosomal dominant pattern of transmission, with variable degree of penetrance. The age-related penetrance for this group of families (fig 4) is: <20 years, 10%; 20–30, 34%; 30–40, 60%; >40, 90%. In five families the evidence suggests a recessive trait (2, 5, 6, 11, and 12). All families contain several cases of dilated cardiomyopathy and the large family (1) is of particular interest. Four families (3, 4, 7, and 8) that show a dominant trait have evidence of mildly dilated congestive cardiomyopathy,⁴ with severe systolic dys-

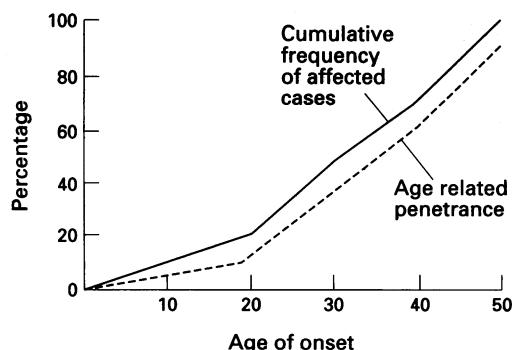
function of the left ventricle and minimal or absent dilatation.

As a first approach to determine the genetic factors responsible for familial dilated cardiomyopathy in these families, we studied the association of several HLA (or major histocompatibility complex) alleles with the disease. HLA has a key role in the regulation of the immune system and an association with HLA-DR4 was found often in patients with dilated cardiomyopathy.⁵⁷

We tested the hypothesis of linkage of the putative disease gene with the HLA region on the basis of HLA class I and II serological polymorphism. Preliminary results in nine families⁵⁸ indicated clear evidence against linkage between the HLA locus and the disease. We concluded that genes of the HLA region do not have a primary role in determining the inheritance of familial dilated cardiomyopathy within our families. The study has been extended to other families.

Other candidate genes are being studied by molecular genetic techniques. Genes coding for proteins involved in myocardial function are candidates for study. Contractile dysfunction, autoimmunity, or altered myocardial metabolism are important features in familial dilated cardiomyopathy: therefore, we are testing the hypothesis of linkage of the disease gene with candidate genes coding for proteins of the contractile apparatus, genes involved in

Figure 4 Like many other autosomal dominant diseases familial dilated cardiomyopathy is usually not present at birth and develops later in life (delayed onset). Mean age of onset of this disease is about 30 years.



the immune regulation, and genes involved in metabolic pathways.

For this genomic study we selected a single large generation family of five generations with autosomal inheritance of the disease (fig 3, family 1). The study of this single family (over 60 members) offers the advantages of avoiding any genetic heterogeneity in the putative disease gene and, at the same time, ensures sufficient statistical power.

Many candidate genes have been tested so far in this family, including genes involved in the immune regulation. Figure 5 shows a study of a polymorphism of the HLA locus. This polymorphic region was studied by a microsatellite polymorphism and the polymerase chain reaction. Molecular genetics confirmed the serological results, showing clear evidence against linkage between HLA and familial dilated cardiomyopathy in this family.

Preliminary data reported by Pastores *et al* indicate that another important candidate gene, cardiac β myosin heavy chain gene, the gene involved in hypertrophic cardiomyopathy, is not linked to the disease.⁴²

study the effects of the altered gene product. They also have clinical and therapeutic implications.

Once the molecular defect is established, it will be possible to develop new tests to detect the carriers of the disease gene, particularly children, in whom the disease may not be clinically evident. Moreover, early detection of the disease may enable the prevention and treatment of the major complications, such as heart enlargement, heart failure, arrhythmia, and sudden death, and lead to appropriate genetic counselling.

Finally, new treatments, such as gene therapy, could be developed to modify the pathogenetic mechanisms causing the disease and not simply the symptoms.

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Conclusions

The identification of frequent genetic transmission of dilated cardiomyopathy provides an important tool for the study of the pathogenesis of this disease, which is a frequent cause of admission to hospital and of heart failure, and the most frequent indication for heart transplantation. Molecular genetic techniques can identify the gene causing familial dilated cardiomyopathy and can be used to

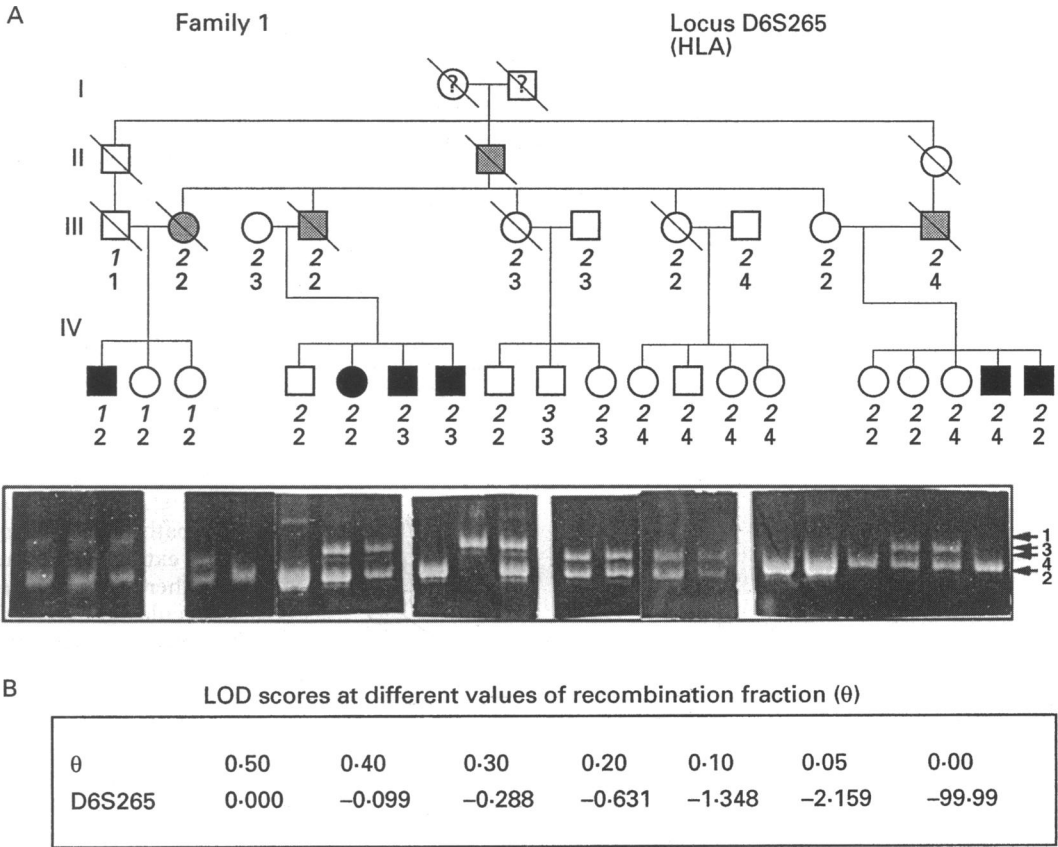
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Figure 5 Segregation of the microsatellite sequence polymorphism AFM184xa11 (locus D6S265), located within the HLA region, in the pedigree of family 1. (A) Segregation of the alleles in the "key" family members (upper part). Ethidium bromide staining of the products of amplification of the microsatellite locus D6S265 after polyacrylamide gel electrophoresis: 4 different alleles are shown. Transmission of the disease and transmission of the genetic marker are completely dissociated. (B) Linkage analysis for the cosegregation of AFM184xa11 with the disease in family 1: the LOD score indicates that linkage can be excluded at a distance of 5 cMorgans (5×10^6 base pairs) from the marker.



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